Memo

Frederick C Mills 8/27/01

Date: Aug. 27, 2001

From: Frederick C. Mills, Staff Scientist, DTP, OTRR, CBER Barry Cherney 8/27/01

To: Amy Rosenberg, M.D., Director, DTP, Barry Cherney, Ph.D., Deputy Director, DTP
cc: Serge Beaucage, Ph.D., DTP, Gary Kikuchi, Ph.D., DTP, Lori Tull, R.N. DARP, OTRR, Jeanne Delasko, R.N., DARP, OTRR
Cc : file

Subject : BLA STNL103951
NESP (Novel Erythropoesis Stimulating Protein) for treatment and prevention of anemia in end-stage renal disease

Manufacturer: Amgen, Inc.

Specific Topic: CM & C review of BLA STNL103951.

This review contains:

1. A review of the original BLA submission for NESP expression construct, cell banks, viral validation, viral clearance, drug substance comparability, intermediate products, drug product, and selected methods validation.
2. A review of Amendment 12 (a major amendment, arising from the August 31, 2000 CMC teleconference)
3. A review of Amendment 16, which deals with quantitation of Northern blots.
4. The February 16, 2001 complete response letter
5. A review of Amendment 26, which is a response to the February 16, 2001 CR letter
6. Minutes of the April 23, 2001 CM & C teleconferences, in which minor information requests arising from review of Amendment 26 were discussed.

For further review of the BLA and Amendment 12, including review of NESP production culture, purification, drug substance characterization and lot release, and the majority of method validations, see the review by Dr. Serge Beaucage, DTP, OTRR. For discussion of the immunogencity assay, see the review by Dr. Gary Kikuchi, DTP.

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I. Cell Banking

Summary of the NESP Expression Construct

NESP has two additional N-linked glycosyl groups relative to ← for a total of five N glycosylation sites. This was accomplished by (-

Validation of the [redacted] production plasmid.

Summary of Issues Regarding the NESP expression construct

This section is straightforward and complete. There are no reviewer's comments on this section

Creation and validation NESP Cell Banks

The [redacted] production plasmid was

Summary of Genetic stability of the CHO NESP cell line during storage and production.

1. Northern, Southern blotting, and sequencing of the NESP product gene was performed on the MCB, WCB, EPC, and cells culture beyond the normal number of generations for a production run.
2. Viability, growth, and Population Doubling Levels (PDLs) were determined as part of the process validation [redacted] to ensure consistent process performance.
3. Cell density and viability are monitored for each thawed vial prior to production to ensure manufacturing consistency.

4. The NESP protein has been completely sequenced from EPCs

Master Cell Bank (MCB)

Question 1. *Please describe the security measures that prevent mixing-up MCB (the current MCB) with other MCB clones.*
Summarizing the response in Amendment 12:
Amgen describes adequate procedures for tracking individual MCB vials, which involve a unique identification number for each vial. Vials are a stored in dedicated canisters under the control of a Cell Bank manager, in a secure, access-controlled warehouse.

Screening of MCB for viral and microbial contamination

MCB was extensively screened for endogenous and exogenous viral contaminants, sterility, mycoplasma, and species identity. Except for sterility testing, which was done at Amgen, all testing was done at

1. Thin section EM

Cells from MCB were fixed, sectioned and examined by EM. At least 100 cell sections, selected in a manner that a high proportion of these cells originated from different individual cells, were examined at > 50,000 magnification. The cells were evaluated for the presence or absence of viral particles, and in particular, for type A and /or type C retroviral particles. There was no evidence for viral particles by this assay. (*see further discussion below on for retroviral particles observed in negative staining on End of Production Cells*)

2. Inoculation into animal models

Embryonated eggs were inoculated by several routes. Allantoic and yolk sac injections allow detection of orthomyxoviriuses (influenza), and paramyoxviruses (parainfluenza, mumps, and measles), Herpes viruses, rickettsiae, myoplasma, and bacteria. Chorioallantoic membrane and amniotic cavity injection allow for detection of HSV, vaccinia, and variola virus.

Inoculation of suckling mice allows for detection of Togavirus, Bunyavirus, Flavivirus, Picorna viruses, and HSV. Guinea pigs were inoculated to allow for detection of paramyxovirus (Sendai), and reoviruses. Adult mouse injections were performed to allow for detection of coxsaki virus and Flavivirus. All of these animal model assays gave no evidence for viral contamination.

3. Cocultivation with *Mus Dunni* cells

Mus dunni cells support replication of xenotropic, amphotropic , MCB, and ecotropic murine leukemia retroviruses. *Mus dunni* cells were cocultured with MCB— cells for five passages. A —— assay was used to test for infection retrovirus. These assays gave no evidence of retroviruses.

4. In vitro viral tests

Supernatants from MC — cells were incubated with MRC-5 (HuEK line), VERO, CHO K1, Bovine Turbinate, and NIH 3T3 cells. This panel of cell lines will detect Picornavirus (poliovirus, coxsakivirus A, B, echovirus, rhinovirus); Orthomyxovirus (influenza); Paramyxovirus (parainfluenza, mumps, measles) Herpesevirus (HSV and CMV) Adenovirus, and Reovirus. These assays were negative for viral contamination.

25. MCB — cells were found to be negative for Mn^{+2} and Mg^{+2} dependent reverse transcriptase activities. This is an assay performed on MCB medium by —— —— ' —— . A similar assay on lysed cells would yield a signal ~ 2X above background.

MCB — cells were analyzed for isoenzymes and found to possess a pattern consistent with CHO cell origin. They also express hamster cell surface antigens. MCB ampules are stored in limited access facilities in multiple, geographically different locations to insure safety of supply.

Working Cell Bank (WCB —

An ampule from MCB — was thawed and first expanded as an adherent culture, and then further expanded in suspension culture in spinner flasks. Ampules containing ~ — cells were frozen to create — ampules of WCB— During the MCB to WCB expansion, the cells underwent — population doublings. Because each MCB ampule can generate —— ampules, a long-term supply of product is guaranteed.

Tests on WCB —

1. Sterility testing: performed or— andomly selected vials. There was no microbial growth, and samples met USP sterility test requirements.
2. Mycoplasma: DNA staining and agar and liquid culture. There was no staining and no evidence of growth.
3. In vitro viral cultures: Utilized a panel of five cell lines as per item MCB item 4. There was no evidence of viral growth.

The growth characteristics of WCB —L were confirmed by ——— —— analysis as per the MCB. (Tables IIC-8 and IIC9) The WCB — ampules were divided among multiple LN2 Dewars for storage. Like the MCB, WCB ampules are stored at geographically different locations. As Minor Question 2 from the 8/31/2000

teleconference Amgen was asked to provide specifics on the geographically distinct locations; i.e.

Minor Question 2. *Please provide specifics on the geographically distinct storage locations of MCB- and WCB*

Summarizing the response in Amendment 12:

The MCB is stored in at Thousand Oaks, while MWCB vials are stored also stored in and at Thousand Oaks, Amgen and the

End of production cells

EPC from three lots were used: One GLP run used to manufacture material for toxicology studies and two GMP runs.

1. In vivo viral testing: Study reports for protocol numbers C30193.03
2. Mycoplasma-DNA staining and agar and liquid culture. No staining and no evidence of growth.
3. Negative Stain EM (Study reports for Protocol number C30022.04) Two lots gave evidence of particles at the detection limit of the assay (1.3×10^6 particles/ml of test sample), while a third lot was negative. Amgen states that they

In the issues communicated to Amgen in the 8/31/2000 teleconference, this was addressed by minor point 6., i.e.

6. *Please provide original figures for the negative staining electron microscopy for the End of Production Cells (Study reports for Protocol number C30022.04)*

Original figures were supplied in Amendment 12, and judged by Dr. Rona Leblanc, DTP, to demonstrate viral particles.

4. Inoculation into Pathogen -Free Mice: check for anti-viral antibodies
   a. per os-enteric viruses in alimentary canal
   b. intranasal
   c. intraperitoneal

After 28 days blood was collected and antibodies to 15 different viruses were measured in serum (ELISA or IFA: Table II C-7, specific procedural details are found in Positive control sera gave appropriate virus-specific reactivity for each virus, and negative control mice were negative for antibody to the virus for ELISA or IFA was performed. These assays were negative for mice inoculated with End of Production Cells.

5. In vitro viral cultures-panel of five cell lines as per item MCB item 4. There was no evidence of viral growth.

6. Growth characteristics

Confirmed requirements for growth, Ability to grow on HT medium, Retarded growth and killing at concentrations higher than which is the highest concentration at which the cells were selected.

Genetic stability of the NESP cell banks.

Southern Blots

DNA was analyzed from MCB — WCB — WCB — EPC, WCB — ., CHO DFR⁻ cells (negative control), CHO DFR⁻ cell DNA spiked with —NESP at —— Blots were hybridized with seven different probes:

——NESP vector DNA, —— NESP cDNA, and oligos for (1) 5' UTR, (2) 5' end of NESP coding sequence, (3) 3' NESP UTR, (4) Internal position in the DHFR minigene, (5) 3' position in the DHFR minigene.

In addition to the expected bands arising from the intact NESP production construct, two rearranged species were detected

Explanations of rearranged species (pertains to both Southern blots-above, and Northern blots-below):

The first rearrangement is a —— from ——

A second rearrangement is a —— from

In minor question 3, from the 8/31/2000 teleconference, Amgen was asked for quantitative analysis of the Southern blots; i.e.

Minor Question 3. *Regarding the Southern Blot data used to characterize the transfected NESP constructs in MCB, WCB, and EPC, it is stated that there were no differences in comparing the hybridization patterns among the different cell banks, as well as EPC and EPC+16 cells. In order to support this conclusion, please supply quantitative analysis of the Southern blots.*

Summarizing the response in Amendment 12:

In Minor Question 4. from the 8/31/2000 teleconference Amgen was asked specifically about transcripts from the rearranged construct lacking a promoter i.e.;
Minor Question 4. *In the MCB — cells, — NESP constructs have been*

Northern Blots

Northern blots were performed on RNA from MCB —, WCB —, WCB— EPC, and EPC+ — generations. Probes used on the Northern blots were — NESP vector DNA, — NESP cDNA, 3' NESP UTR, Internal position in the DHFR minigene, 3' position in the DHFR minigene. Amgen states that there were no differences in the RNA bands between any of the cell sources with any of the probes.

However, the Northern blots that are presented in the BLA (probed with NESP cDNA and the probe from the 3' part of the DHFR minigene) show [redacted] loading and considerable [redacted] This makes the relative amount of NESP or DHFR transcript impossible to interpret.

In Minor Question 5 from the 8.31.2000 Amgen was asked to provide quantitative data on hybridization intensities; i.e.

Minor Question 5. *Northern blots were performed on RNA from MCB [redacted] WCB [redacted], WCB [redacted] EPC, and WCB [redacted]* [redacted]

Subsequent to the submission of [redacted] Amgen submitted [redacted] which describes quantitation of Northern blots. For this communication a series of Northern blots were performed, and the autoradiographs were analyzed by densitometry.

Summarizing this communication:

Sequence verification of the NESP production construct and rearranged constructs

DNA Copy Number of NESP expression constructs

Viral testing on Cell Banks and EPCs
Testing of Master Cell Bank

Consider . Six photographs of sections are attached. There was no evidence of retroviral particles.

Viral Testing on Working Cell Bank

Sterility-USP
Mycoplasma

Viral testing on End of Production Cells

Mycoplasma testing as per WCB

WCB — cells were shown to be negative for mycoplasma.

As part of the 8/31/2000 teleconference, Amgen was asked for further information on the EPC cells; i.e.

Minor Question 7. *You have provided extensive mycoplasma and viral testing data for three EPC lots. Please provide the lot numbers for NESP product derived from these EPC lots.*

The lots used were

EPC Lot #	Purified Bulk lot #	Final Dosage Form Lot #
1.	non-GMP	
2.		µg/ml
		µg/ml
3.		µg/ml
		µg/ml
		µg/ml
		µg/ml

Negative staining EM was performed on EPC cells There are three separate reports for three different batches of EPC cells. Testing was performed by

Minor Question 6. *Please provide original figures for the negative staining electron microscopy for the End of Production Cells (Study reports for Protocol number*

(30022.04) Satisfactory original figures for negative staining EM on the EPC were supplied, were reviewed by Dr. Rona LeBlanc, and judged to show viral particles

What production batches were used for these electron micrographs?
See response to Minor Question 7, above.

There needs to be clarification of how these samples were prepared. Was there a

Mouse antibody tests on End of Production Cells

1. Security measures for the cell banks were described in Amendment 12 (Minor Question 1)

2. Specifics on geographically distinct storage of the Cell Banks have been satisfactorily described in Amendment 12 (Minor Question 2).
3. Quantitative Southern blot data for the MCB, WCB, and EPC have been supplied in Amendment 12 and show satisfactory stability (Minor Question 3).
4. As requested, the sensitivity of the Northern Blot analysis was described in Amendment 12 (Minor Question 4).
5. Adequate quantitative Northern blot data were supplied in Amendment ---, submitted November 13, 2000. (Minor Question 5).
6. Satisfactory original figures for negative staining EM on the EPC were supplied, reviewed by Dr. Rona LeBlanc, and judged to show viral particles (Minor Question 6)
7. The numbers of three lots used for extensive viral and mycoplasma testing were supplied in Amendment 12 (Minor Question 7).

Summary
Adequate characterization of the cell banks has been supplied and there are no outstanding issues.

II. Major CM & C issues

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These limits would appear to supply adequate control over bioburden at the important stages of NESP manufacturing

In response to the second part of Major CM & C issue 1., Amgen has supplied the data

Minor Question 10. *It is mentioned* [redacted] *that lot* [redacted] *was discontinued*

Additional clarification on the microbial control of the NESP manufacturing process was obtained in the response to Minor Question 8 from the 8/31/2000 teleconference; i.e.
Minor Question 8. *Please describe in more detail how the multiple steps involved in NESP , can consistently be achieved under aseptic conditions.*

Overall, the information provided in the BLA and Amendment 12 indicates satisfactory microbial control of the NESP cell culture. NESP from lot has apparently not yet been formulated for clinical use, since IND

(July 13, 2001) describes the most recent drug product and pertains to formulation from lot

2. Routine in process viral testing on

An additional viral concern regarding the production cell culture was the use of porcine trypsin, as discussed as Minor Question 9 during the 8/31/2000 teleconference; i.e.

Minor Question 9. *Please provide data demonstrating that the porcine trypsin used in scale-up to is free of porcine retrovirus.*

Amgen uses Trypsin from irradiates the Trypsin at 25 to 40 kGY to destroy viral contaminants. The product is then tested for porcine parvovirus in accordance with 9 CFR Part 113.53. Amgen tests every lot of trypsin for

sterility upon receipt and for porcine parvovirus once a year. This response was also reviewed by Dr. Rona LeBlanc, DTP.

Major Question 3: EM data for retroviral burden

Please provide data on the retroviral burden [redacted] that is representative of the manufacturing process. We suggest the use of negative staining EM. A response to this request should include a detailed description of the methods used.

[redacted]

"Unprocessed bulk supernatant concentrates or ascites should be assayed prior to any manipulation other than clarification by low speed centrifugation, unless it can be shown that virus testing would be made more sensitive by initial partial processing"
FDA, Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human use, General consideration on quantification and removal of a retrovirus contaminant, Section II.C.4 (1997)

From the experience with [redacted] the lot-to-lot variation in retroviral counts is greater [redacted] step for [redacted] does not

[redacted]

staining. Negative staining does not permit identification of viral morphology, so that [redacted] Amgen also sites FDA points to consider: i.e.

"The amount of retrovirus in the unprocessed bulk should be quantified on a series of bulk harvests and shown to be consistent from lot to lot. Endogenous virus particle burden should be determined at the end of a typical fermentation, prior to purification process, preferably by thin section EM on material pelleted by ultra centrifugation.
FDA, Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human use, General consideration on quantification and removal of a retrovirus contaminant, Section II.C.4 (1997)"

Amgen reports the presence of retrovirus-like particles in

[redacted]

Lot ( )	Particles detected	Particles per ml	Average per lot Particle per ml
	Mean		
Maximum			

The retroviral burden appears to be consistent, and small enough to not pose a safety issue, after clearance by the NESP manufacturing process (see below- **Comparison of Viral clearance on new and reused columns**)

Major Question 4. Column resin reuse validation

For columns please provide data demonstrating the effectiveness of these process steps with respect to all of the columns' intended functions. It is important that this validation data should include information on the clearance model viruses from each of column

A summary table of column chromatography parameters for lab scale vs. commercial scale is found in Amendment

Column

Volume

Column
Volume

Column
Volume

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Pages 27-29

(Amendment 12,

A calculation of was performed using an average

Summary of Issues Regarding Responses to Major Questions Raised in the 8/31/00 teleconference.

Major Question 1. Routine in-process bioburden testing

Minor Question 8: Aseptic conditions during

Minor Question 10: Discontinuation of Lo

Minor Question 12: TSA and SBA for enumeration of total microbial counts

In Amendment 12, the checkpoints, alert limits, and action limits for bioburden testing during the NESP process are adequately described in tabular form. Precautions to maintain aseptic conditions during scale-up and discontinuation of Lot , and volume collected for measurement of total microbial counts, as well as bioburden of the medium, are adequately summarized.

Major Question 2. Routine in-process viral testing

Minor Question 9.: Porcine trypsin, lack of retroviral contamination

Amendment 12 provides an adequate description of viral testing

Major Question 3. EM data for retroviral burden of

Amgen supplies an adequate rationale for EM analysis on

Major Question 4. Column resin reuse validation

III. Comparability of NESP Drug Substance After Scale-Up

During the course of product development, the NESP process has undergone several scale-up operations. The scale-up operations that involve product used

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Summary of issues regarding intermediate products

Amgen has provided satisfactory responses for the following Minor questions arising from the August 31, 2000 CM & C teleconference:

Minor Question 13. IEF gels show even though specification is for bands
Response: gels are shown to which has large range

Minor Question 14 Decision process for deciding if peptide map

Response: Satisfactory criteria for the provided.

Minor Question 15. Shipping procedure for the Purified Bulk
Response: Shipping procedure described in satisfactory detail

Minor Question 16. Request for long-term stability data on more the one lot of Purified Bulk.
Response: 24 month data for two additional lots provided

Minor Questions 17. Effect of

Response: Comparison of NESP samples first treated

Oustanding issue:
The suggestion that
Amgen.

V. Final Product

Polysorbate Formulation

Specifications for the Polysorbate Formulation Final Product

Identity

SDS-PAGE

Western blot

The acceptance criteria for this specification were clarified via Minor Question 20 from the 8/31/2000 teleconference; i.e.

Minor Question 20. *For both Polysorbate and Albumin formulations, the acceptance criterion for Western Blots of SDS PAGE gels is given as " ". Please provide the decision process used to determine whether the drug product is within specification.*

IEF

Western blot

The acceptance criteria for this specification were clarified via Minor Question 22 from the 8/31/2000 teleconference; i.e.

Minor Question 22. *For lot release of the polysorbate final product, the SDS-PAGE criterion is and the IEF criterion is provide the decision process used to deter*

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Minor Question 21. *For the albumin formulation, the specification for SDS-PAGE . Please provide the decision process used to determine whether the albumin formulation is within specification.*

The main band of the test sample must have the same mobility as the main band

The SDS-PAGE criterion is — '. IEF is — ' There should be a clarification of the decision processes for these assays These issues were the subject of extensive discussion, summarized in the minutes for the CM & C teleconference held April 23, 2001, 1:00-1:30 p.m. The minutes found at the end of this review.

It appears that no — are shown in the BLA for the albumin formulation, and no — ——— are shown for either formulation. A request for this information constituted Minor Question 22 from the 8/31/2000 teleconference; i.e

Minor Question 19. *Regarding drug product stability data, —— are shown for the —— formulation and no ——— are shown for either the polysorbate or —— formulations. Please provide these data.*

Response provided in Amendment 12:

Photographs of albumin formulation SDS/PAGE — were provided in ——— ——— .. Due to interference from albumin, ——— nalyses were not an integral part of the stability program for the NESP albumin formulation. ——— of NESP polysorbate stability samples are provided. On chromatographs with an

expanded vertical scale, ——— material is visible for NESP polysorbate drug product kept on stability for 21 months. Shown are chromatographs for one —— formulation, one —— formulation, and ——— formulations.

There was a —— for — polysorbate lots at the highest concentration —— to show a ——— —— of starting value. This is not reflected in the in vitro bioassay. Figure IIF-36, p. 125 ——————— ——— This issue was addressed in Minor Question 26 from the 8/31/2000 teleconference; i.e.

Minor Question 26. *There was a trend for — polysorbate lots at the highest concentration ——— to show a ——— to —— of its initial value. This is not reflected in the in vitro bioassay. Figure IIF-36, p. 125 suggests the occurrence of some ——— Please clarify the mechanism of this* ——

In Amendment 12, Amgen cites as an explanation the variability of the NESP ———, in which an observed Coefficient of Variation (C.V.) c — is seen with the —— —— stability samples, which is comparable to the — C.V. obtained for the assay controls. Furthermore, the % area recoveries for ——— on ——— stability samples were consistently above — arguing against a decline in —— concentration.

Minor Question 23 *Please provide any available stability data on ——— n —*

In Amendment 12, it was stated that Amgen has an ongoing program to assess the —— ——— of the NESP —— —— with samples stored at —— for 19 months having been analyzed. To date, there are no significant differences in the rate of ——— ——— between NESP vials and —— of similar concentrations.

Acccierated stabilitv for the polysorbate formulation

Photostability (both formulations)

Minor Question 24. *Please provide a description of the* [redacted] *used in the photostability studies, including the* [redacted]

Minor Question 25. *There are* [redacted] *Photostability data on* [redacted] *or alternatively, a convincing argument should be made as to why their photostability properties would be similar to those of vials. A* [redacted] *should be provided, so the FDA can assess the light-blocking properties of these packs. In addition, please provide a dispensing pack and distribution pack for NESP vials.*

Response in Amendment12 :

As discussed during the September 5, 2000 teleconference, photostability studies of NESP in Type 1 [redacted] glass vials documented that the product is light sensitive.

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Equivalence of polysorbate and albumin formulations

Amgen has documented equivalence of in vitro cell proliferation activity for polysorbate and albumin formulations at — and —. NESP in both formulations shows — Moreover, studies demonstrating bioequivalence in both beagle dogs and healthy male volunteers have been performed.

Challenge of — for leaks (Container closure- — were challenged using — test. This was performed for both — and vials at APR (Amgen Puerto Rico) — vials were filled under aseptic conditions with — Following initial — and assessment for sterility, each lot was divided into three groups:

Group 1: — vials cycled between -70 ^{0}C and RT every 24 hours for 3 consecutive days.

Group 2: — vials cycled between 37 ^{0}C and 4 ^{0}C every 24 hours for 3 consecutive days.

Group 3: — vials stored long term in a horizontal position at 2-8 ^{0}C.

All containers met the initial testing criteria. Samples from the remaining long-term groups were scheduled for annual inspection for the duration for these studies (60 months). All containers to date have passed inspection at 12 months.

Summary of issues regarding the Final Product

Amgen has supplied satisfactory responses to the following Minor Questions arising from the September 31, 2000 CM & C teleconference:

Minor Question 18. Where will the — be performed?
Response: Amgen Puerto Rico

Minor Question 19 — for the stability samples
Response: Photographs of albumin formulation SDS/PAGE — were provided in Part —

Minor Question 20. Clarification of —

Response: —

Minor Question 21. Clarification of — samples —

Minor Question 22. For Drug Product stability, no SDS-PAGE [redacted] are shown for the HSA formulation and non [redacted] are shown for either formulation
Response: [redacted] are shown for the albumin formulation in the BLA. [redacted] is not a part of stability program for albumin formulation due to interference form albumin. [redacted] for polysorbate formulation were provided in Amendment 12.

Minor Question 23. Provide any available stability data for [redacted] formation in [redacted]
Response: There is an ongoing stability program for [redacted] formation, with samples at 19 months being analyzed. There were no significant differences in the rate of [redacted] between vials and [redacted]

Minor Question 24. Provide a description of [redacted] used in photostability studies
Response: Satisfactory description provided

Minor Question 25. [redacted] f photostability data for [redacted] request for [redacted]

Response: [redacted] are made of the same [redacted] glass [redacted] therefore photostability data on [redacted] would appear to be unnecessary. [redacted] been provided and appear to provided satisfactory light protection.

Minor Question 26. There was a [redacted] at the highest concentration [redacted] to show a [redacted] concentration [redacted] of its initial value.
Response: Amgen cites as an explanation the variability of the NESP [redacted] assay as well as the fact that the % area recoveries for [redacted] stability samples were consistently above [redacted] arguing [redacted] concentration.

There are no outstanding issues regarding the Final Product

VI. Methods Validation

The following analytical methods were reviewed by Dr. Mills

[redacted]

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VII. February 16, 2001 complete response letter and subsequent actions

Complete Response Letter: February 16, 2001

Our STN: BL 103951/0

George Morstyn, Ph.D.
Amgen, Incorporated
One Amgen Center Drive
Thousand Oaks, CA 91320-1789

Dear Dr. Morstyn:

This letter is in regard to your biologics license application for darbepoetin alfa submitted under section 351 of the Public Health Service Act. Reference is also made to our teleconference dated September 19, 2000, between representatives of Amgen and CBER, and your response dated October 2, 2000. Reference is also made to our December 15, 2000 Discipline Review letter.

The Center for Biologics Evaluation and Research (CBER) has completed the review of this application. Our review finds that the information and data submitted are inadequate for final approval action at this time based on the deficiencies outlined below.

Chemistry, Manufacturing, and Controls Section:

1. The _____ presented in the BLA for ____ of darbepoetin alfa drug product and ____ of bulk drug showed a range of _____ of the _____ Moreover, the method validation for the *in*

_____

_____

Please submit all data supporting your proposed specifications.

2. Regarding drug substance testing and specifications

   a. In accordance with the International Conference on Harmonization document Q6B entitled, *Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products* (available at http://www.ifpma.org/ich5q.html), please institute a lot release specification for _____ manufacture, and submit data supporting your proposed specification.

   b. As described in your October 2, 2000 submission, the specification for the SDS-PAGE _____

c. Please institute specifications for the relative intensities of the bands observed _____ and submit data supporting your proposed specification in your response.

d. Please develop a lot release specification for the minor _____ darbepoetin alfa and submit data supporting your proposed specification in your response.

e. Please define the phrase "Conforms to Standard" (CTS), in regard to the _____ specification.

f. Please revise the Certificate of Analysis (COA) for bulk drug substance to be in accordance with the above changes, and submit a copy of the revised COA.

3. Regarding immunogenicity of the drug product:

a. The current assay for antibodies to darbepoetin alfa is not sufficiently sensitive, _____

improved quantitative ability. Prior to using the new assay, we request you submit the validation data to your IND for review.

b. We request that you use this assay to re-test archived serum samples from patients in the clinical trials. Please submit the results and revised draft labeling.

c. You submitted information on immunogenicity of darbepoetin alfa in a formulation containing albumin but not the polysorbate-containing, albumin-free formulation. Please provide information on the immunogenicity of the polysorbate formulation of darbepoetin alfa using the new assay. Please submit revised draft labeling.

d. In the event the new assay detects antibodies to either formulation of darbepoetin alfa, _____ darbepoetin alfa a.....

Steps to address the above issues should be initiated now, but may be completed with postmarketing commitments. Please describe your plans to address each of these four issues in sufficient detail to permit our evaluation of the adequacy of the proposals. We request that your response include:

- a proposed schedule for developing and validating each assay and submitting the results to CBER;
- a description of each study, including numbers of serum samples to be tested; and,
- a schedule for conducting each study and submitting of the final study report and applicable revised labeling to the CBER.

4. Please submit validation summaries from three consecutive, successful sterilization runs for all equipment used for the aseptic filling and support operations for the formulation and filling of darbepoetin alfa. These summaries should include, but not be limited to, the following information:

5. Please submit a narrative description of the viable and non-viable environmental monitoring program for class 100 environmentally classified areas at both the Thousand Oaks, California and Juncos, Puerto Rico locations. The information should include the frequency of environmental monitoring; locations monitored; alert and action levels; descriptions of actions taken when alert and action levels are exceeded; and, information on the monitoring program for yeasts and molds.

6. Please provide validation summaries of testing performed supporting product compatibility and microbial retention for the sterilizing [redacted] used in the [redacted] stage at the Juncos, Puerto Rico location.

Clinical Section:

7. Preliminary comments regarding our review of the clinical section of your application were communicated in our Discipline Review letter dated December 15, 2000. In preparing your complete response, please ensure you completely address each deficiency delineated in our December 15, 2000 letter. We acknowledge receipt of your December 28, 2000, submission. You may cross reference applicable sections of that amendment in your complete response to this letter and those sections will be reviewed as part of your complete response.

8. As noted in our Discipline Review letter dated December 15, 2000, the darbepoetin alfa safety database raises concern regarding enhanced susceptibility of patients of African descent to darbepoetin alfa induced hypertension. As described in that letter, we request that you conduct a postmarketing study to further evaluate the risk of hypertension in subjects of African descent. We also requested additional pediatric studies. Please describe your plans to address these issues in sufficient detail to permit our evaluation of the adequacy of the proposals. We request that your response include:

   - A detailed protocol or, at a minimum, a detailed outline describing all design features of the study including sample size and justification, eligibility criteria with rationale, dosing regimens and duration, clinical assessments to be performed and their timing, and endpoints to be analyzed.

   - Proposed schedule for conducting the study, including all major milestones for the study (e.g., submission of finalized protocol to the FDA, completion of patient accrual, completion of the study, and submission of the final study report, SAS dataset and applicable revised labeling to the FDA).

   Please be advised that submission of complete protocols for review and comment should be submitted to your IND and may be cross-referenced in your response to this letter.

9. As discussed during the telephone conversation of February 2, 2001, between Ms. Cheryl Anderson and Ms. Nancy Picarello of Amgen, and Dr. Ellis Unger of this office, we understand that you are planning to revise reported rates of adverse events for incorporation in the package insert. Please submit a revised table of adverse events for the proposed package insert, including all events with an incidence of 5% or greater in darbepoetin alfa-treated subjects.

10. Darbepoetin alfa, like other products in this class, is likely to be self-administered by some patients. Therefore, please submit a draft patient information sheet for the product. We request that this label provide information, in a question and answer format, about risks as well as steps for preparation and administration.

We have considered your proposed trade name in consultation with CBER's Advertising and Promotional Labeling Branch and have no objection to your proposed trade name "ARANESP" at this time. However, a formal acceptance of your proposed trade name cannot be given at this time, since another product with a similar name (e.g., sound-alike or look-alike) could be approved prior to the approval of your product.

We reserve comment on the proposed labeling until the application is otherwise acceptable.

You may request a meeting or teleconference with CBER to discuss the steps necessary for approval. Should you wish to have such a meeting, please submit your meeting request as described in the FDA Guidance for Industry: Formal Meetings with Sponsors and Applicants for PDUFA Products – February, 2000 (http://www.fda.gov/cber/gdlns/mtpdufa.pdf).

Within 10 days after the date of this letter, you are requested to take one of the following actions: (1) amend the application; (2) notify us of your intent to file an amendment; (3) withdraw the application; or (4) request an opportunity for a hearing on the question of whether there are grounds for denying approval of the application. In the absence of any of the above responses, CBER may initiate action to deny the application.

Please note our review clock has been suspended with the issuance of this letter. Note also that any amendment should respond to all deficiencies listed and that a partial reply will not be considered for review nor will the review clock be reactivated until all deficiencies have been addressed.

Should you need additional information or have any questions concerning administrative or procedural matters please contact the Regulatory Project Manager, Jeanne Delasko, in the Division of Application Review and Policy at (301) 827-5101.

Sincerely yours,

Karen D. Weiss, M.D.
Director
Division of Clinical Trial Design
and Analysis
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Amy S. Rosenberg, M.D.
Director
Division of Therapeutic Proteins
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

cc: STN 103951/0 file
HFM-588/J.Delasko
HFM-588/L.Tull
HFM-541/F. Mills (comments rec'd 2/6 01)
HFM-541/S.Beaucage
HFM-541/B.Cherney
HFM-541/G.Kikuchi (comments rec'd 1/26/01)
HFM-538/A. Rosenberg
HFM-576/E. Unger (comments rec'd 2/13/01)
HFM-579/D. Green (no comments)
HFM-579/M. Serabian (no comments)
HFM-570/K. Weiss
HFM-570/P. Keegan
HFM-505/E.Dye
HFM-215/G. Gupta
HFM-650/P. Holobaugh (no comments)
HFM-676/P. Amin (comments rec'd 2/13/01)
HFM-676/R. Darius (comments rec'd 2/13/01)
HFM-585/G.Jones
HFM-576/M. Walton
HFM-602/C. Miller
HFM-4/QAS

CBER:OTRR:DARP:L.Tull:2/13/01:JMDelasko
Revised:2/13/01:dixon:2.14.01:JMDelasko Revised:2/16/01:dixon:2.16.01
(S:/Delasko/Letters/STN103951CR001.doc)

MILESTONE - COMMUNICATION TYPE:
LETTER: Complete Response (CR)

DATA CHECK:
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		DATE	OFFICE	SURNAME	DATE	OFFICE	SURNAME	DATE

Sincerely,

Frederick C. Mills, Ph.D.

Memo

Date: 3/29/2001

From: Frederick C. Mills, Staff Scientist, DTP, OTRR, CBER

To: Amy Rosenberg, Barry Cherney, Serge Beaucage, Gary Kikuchi, Rona LeBlanc

Subject: BLA STN 103951, Amendment 26 (Complete Response)

NESP for treatment and prevention of anemia in end-stage renal disease

Review of Amgen's Complete Response, submitted in response to CBER's CR letter on February 16, 2001. Amendment 26 was submitted on February 21, 2001 and routed from document control on February 26, 2001.

Comments to the file:

Individual Responses to CM & C issues

1. ***In vitro* bioassay**

   The *in vitro* bioassay data presented in the BLA for —— of darbepoetin alfa drug product and — — of bulk drug showed a range of —— Moreover, the method validation for the *in vitro* bioassay demonstrated — accuracy. Nonetheless, the purified bulk and final drug product specifications for the *in vitro* bioassay have been set as —— of the reference standard potency. Please revise the purified bulk and drug product lot release specifications for the *in vitro* bioassay to reflect darbepoetin alfa manufacturing history and the accuracy and reproducibility of the bioassay. Please submit all data supporting your proposed specifications.

   Amgen's Response

Amgen agrees that the *in vitro* potency assay specification limits for —— Purified Bulk and Final Product (albumin and polysorbate formulations) can be —— from the originally proposed specification —— Amgen has submitted data for 109 lots of Final Product and 48 lots of —— Purified Bulk. The data show a range of values of —— of Standard Potency, with 3 standard deviations around the mean giving a

Reviewer's comments

The proposed ——— the in vitro potency assay limits represents a satisfactory improvement in this specification. Amgen should be committed to further ——— of this specification as warranted by additional manufacturing history and improvement of the assay.

2. Regarding drug substance testing and specifications:

a. In accordance with the International Conference on Harmonization document Q6B entitled, *Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products* (available at http://www.ifpma.org/ich5q.html), please institute a lot release specification for ——— groups at the ——— bulk stage of manufacture, and submit data supporting your proposed specification.

Amgen's response

As recommended by the ICH Q6B Guidance, Amgen has validated the peptide mapping method under ———

Reviewer's comments

Amgen agrees with CBER's request to provide an evaluation for the presence of ——— as part of the lot release specifications, as recommend by ICH Guidance Document. The specification ——— This revised specification is shown in "Revised Filtered Purified Bulk Specifications".

b. **As described in your October 2, 2000 submission, the specification for the SDS-PAGE [redacted] includes the criterion that [redacted] However, [redacted] Please provide the acceptance criterion that will be used for instances [redacted]**

Amgen's response:

Amgen has revised the [redacted]

[redacted]

Reviewer's comments

This revised specification provides satisfactory control over the possibility that novel SDS-PAGE bands might appear in lots of darbepoetin alfa.

c. **Please institute specifications for the [redacted] observed in [redacted] and submit data supporting your proposed specification in your response.**

Amgen's response:

Amgen agrees that additional quantitative specifications for the relative intensities of the bands observed in IEF are warranted. [redacted]

Reviewer's comments

This revised specification provides adequate quantitative control over the relative amounts of darbepoetin alfa glycoforms.

d. Please develop a lot release specification for the _____ of darbepoetin alfa and submit data supporting your proposed specification in your response.

Amgen's response

Amgen agrees with CBER's request to institute a specification for _____ _____ This specification is based on data provided for 52 darbepoetin lots. The analytical method and method validation are provided in an Appendix.

Reviewer's comments

This new specification provides needed control over the amount underglycosylated darbepoetin alfa, which comprises the _____

e. Please define the phrase _____, in regard to the _____ elease specification.

Amgen's response

_____

_____

_____

_____

_____

_____

_____

_____

_____

Reviewer's comments

Adequate quantitative criteria have been defined to support _____ for this specification.

f. **Please revise the Certificate of Analysis (COA) for bulk drug substance to be in accordance with the above changes, and submit a copy of the revised COA.**

Amgen's response:

Amgen has revised the COA for bulk drug substance, and this included in the Complete response.

Reviewer's comments

The numerical limits for each test should be included in the COA template.

(The immunogenicity issues summarized below are covered in a separate review by Dr. Gary Kikuchi)

3. **Regarding immunogenicity of the drug product:**

   a. **The current assay for antibodies to darbepoetin alfa is not sufficiently sensitive, because the assay can only detect antibodies to darbepoetin alfa at a threshold level of ——— We request that you design a new assay for detection of darbepoetin alfa antibodies with increased sensitivity and improved quantitative ability. Prior to using the new assay, we request you submit the validation data to your IND for review.**

Amgen's response:
Amgen recognized the need to continuously improve this assay technology, and fully commits this effort as describe below. A multi-step program has been initiated to accomplish this objective. These initiatives include:

In addition, Amgen is investigating alternate assay platforms capable of detecting ——— to improve assay sensitivity. These include:

[redacted]

These data will be provided to the FDA in the INL — annual report.

b. **We request that you use this assay to re-test archived serum samples from patients in the clinical trials. Please submit the results and revised draft labeling.**

Amgen's response:

Amgen proposes submitting new assay validation data to CBER by [redacted] After CBER's review of this data, Amgen will analyze archived serum samples from 500 subjects in the NESP clinical development program as well as 100 CRF subjects treat with the polysorbate formulation. Amgen commits to completing retesting, submission of results, and filing of revised draft labeling by [redacted]

c. **You submitted information on immunogenicity of darbepoetin alfa in a formulation containing albumin but not the polysorbate-containing, albumin-free formulation. Please provide information on the immunogenicity of the polysorbate formulation of darbepoetin alfa using the new assay. Please submit revised draft labeling.**

Amgen's response:

As a post-marketing commitment, Amgen agrees to antibody testing (baseline and post-24 weeks treatment) on 1000 CRF subjects treated with the polysorbate formulation, using the new antibody assay. These results will be submitted on [redacted]

e. **In the event the new assay detects antibodies to either formulation of darbepoetin alfa, it will be critical to establish whether they neutralize darbepoetin alfa and/or cross-react with native erythropoietin. While the neutralizing antibody assay that you have developed demonstrates an adequate sensitivity, specificity and quantitative ability, an assay to evaluate antibody cross-reactivity has not been described. Therefore, if antibodies to darbepoetin alfa are detected, please develop an assay and submit data to establish whether antibodies to darbepoetin alfa cross-react with native erythropoietin.**

Amgen's response

[redacted] will be tested and the data will be submitted to CBER upon completion of the investigation.

Memo

Date: 5/9/01

From: Frederick C. Mills, Staff Scientist, DTP, OTRR, CBER

To: file

Subject: BLA STN 103951, April 23, 2000, 1:00-1:30 teleconference memo
AMGEN's NESP Epo-related product for treatment and prevention of anemia in end-stage renal disease.

Participants:

CBER: Fred Mills, Serge Beaucage, Barry Cherney, Amy Rosenberg
Amgen: Cheryl Anderson, Heather Simmerman, Evryll Swanson, Andreas Kyriacou, Kimball Hall

As was prearranged between Amgen and CBER, this teleconference was initiated at 1:00 p.m. by Amgen to discuss CM & C information requests resulting from CBER's review of the Amgen response (Amendment 26) to the February 16, 2001 CR letter from CBER.

Dr. Mills clarified that this teleconference was an information request. Ms. Anderson asked Dr. Mills to read each question before it was discussed, so that Amgen would have an accurate understanding of the questions. Dr. Mills did this, and discussion followed after each question was read.

1. Regarding Response 2f (revised COA)
The template COA contains no numerical ranges for the specifications. Please supply a revised template that includes these ranges.

Amgen stated that the specifications, numerical assay ranges, and assay results are captured on an Analytical Data Summary form that is included in each batch record and that this form is required to be reviewed by Quality Assurance prior to release.

n.b. A copy of the Analytical Data Summary form is found on page 8 of Amendment 29.

2. Regarding Response 2b (
How does Amgen decide if

Ms. Swanson responded for Amgen. and stated that samples for production lots of darbepoetin alfa are currently run on SDS-PAGE [redacted] with a reference standard.

[redacted]

Dr Cherney responded that this wording sounded OK, but that CBER would defer final decision until the wording was reviewed. Dr. Kyriacou said that the revised wording would be submitted in an amendment.

n.b. The revised wording, as found in Amendment 29, page 3 reads

[redacted]

3. Regarding Response 1e ([redacted] release specifications)
What are the values for SA/N for the lots used in the clinical trials? Are these different from the total manufacturing history?
On April 21, 2001 Amgen submitted an email to Dr. Mills containing a list of all clinical lots, and SA/N number for these lots. Dr. Beaucage asked why one of these lots- [redacted] was not on this list of lots contained in the manufacturing history in Amendment 26. Dr. Simmerman replied that [redacted] was omitted because it was generated in a research lab.

[redacted]

Sincerely
Frederick C. Mills
Frederick C. Mills, Ph.D

Memo

Date: 5/10/01

From: Frederick C. Mills, Staff Scientist, DTP, OTRR, CBER

To: file

Subject: BLA STN 103951, April 23, 2000, 2:00-2:30 p.m. teleconference memo AMGEN's NESP Epo-related product for treatment and prevention of anemia in end-stage renal disease.

Participants:

CBER: Gary Kikuchi, Fred Mills, Amy Rosenberg
Amgen: Cheryl Anderson, Heather Simmerman, Steve Swanson, Ralph Smalling, Brad Maroni, Tom Ulrich, Anna McDermott

As was prearranged between Amgen and CBER, this teleconference was initiated at 2:00 p.m. by Amgen to discuss an immunogenicity information request resulting from CBER's review of the Amgen response [redacted] to the February 16, 2001 CR letter from CBER.

Ms. Anderson began the discussion by asking CBER if a decision had been reached regarding the acceptability of lot release limits for the SA/N values, as was discussed in the previous (1:00-1:30) teleconference. Dr. Mills responded that a decision had been reached. This decision was to allow Amgen to use limits encompassing 4 standard deviations, with a Phase IV commitment to narrow the limits to 3 SDs when sufficient manufacturing history with commercial lots has been accumulated. Dr. Simmerman asked if a manufacturing history of 30 lots would be sufficient. Dr. Rosenberg responded that this proposal seemed satisfactory.

The remainder of the teleconference involved discussion of the following information request:

Regarding Response 3a (new assays for detection of darbepoetin alfa antibodies)

[redacted]

Dr. Kikuchi asked Dr. Swanson to summarize the new immunogenicity assays currently under development at Amgen. Dr. Swanson did this, and stated that these were all assays

[redacted]

Dr. Swanson also stated that the expected sensitivity of assays was in the range of [redacted] serum. Additional information regarding this assay design will be provided in the November 30, 2001 amendment in response to the CR letter.

Ms. Anderson summarized the discussion, and the teleconference was concluded.

Sincerely

Frederick C. Mills

Frederick C. Mills, Ph.D.

Summary of Review Status as of August 27, 2001

The CM & C review of this BLA has been completed. Amgen has undertaken satisfactory Phase IV commitments in response to the CM & C issues raised in the FDA's February 16, 2001 Complete Response Letter, and the teleconferences held on April 23, 2001. T

Sincerely

Frederick C. Mills

Frederick C. Mills, Ph.D.